Short Communication

Prevention of Topical Surfactant–Induced Itch-Related Responses by Chlorogenic Acid Through the Inhibition of Increased Histamine Production in the Epidermis

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Abstract. Effects of chlorogenic acid on surfactant-induced itching were studied in mice. Topical application of sodium laurate increased hind-paw scratching, an itch-related response, 2 h after application, which was inhibited by topical post-treatment with chlorogenic acid. Sodium laurate increased the histamine content and 53-kDa l-histidine decarboxylase in the epidermis, which were also inhibited by post-treatment with chlorogenic acid. These results suggest that topical chlorogenic acid is effective in the prevention of itching induced by anionic surfactants. The inhibitory activity of chlorogenic acid may be due to the inhibition of an increase in histamine in the epidermis.

Keywords: chlorogenic acid, sodium laurate–induced itching, histamine in keratinocyte

Surfactants used in soaps and shampoos often cause itching (1, 2), a sensation that provokes the desire to scratch. Recently, we have found that a topical application of the anionic surfactant sodium laurate, but not the neutral surfactant N-lauroylsarcosine sodium salt, increases hind-paw scratching, an itch-related response, 2 h after application (3). The effect of topical sodium laurate is suppressed by an H1 histamine–receptor antagonist and is attributed to histamine released from the epidermal keratinocytes (3). Mast cell histamine is not involved in this itching (3).

Natural products are better than synthetic medicines for the prevention of itching due to toiletries. Extracts of plants of Prunus species, especially cherry trees, have several pharmacological effects, including anti-inflammatory, anti-hyperlipidemic, anti-hyperpigmentation, and antioxidant effects (4, 5). It has been recently reported that systemic administration of chlorogenic acid, a main constituent of cherry leaves, exerts weak inhibitory activity on hind-paw scratching induced by histamine and plasma extravasation induced by passive cutaneous anaphylaxis in mice (6). Therefore, in the present study, we examined the effects of topical application of chlorogenic acid on sodium laurate–induced scratching and the mechanisms of anti-pruritic activity of topical chlorogenic acid.

Male ICR mice (7 – 8-week-old; Japan SLC, Shizuoka) were used. They were housed in a room under controlled temperature (21°C – 23°C), humidity (45% – 65%), and light (lights on 07:00 – 19:00 h). Food and water were freely available. Procedures used in the animal experiments were approved by the Committee for Animal Experiments at the University of Toyama.

Sodium laurate (Nacalai Tesque, Inc., Kyoto) was dissolved in distilled water. Chlorogenic acid (Cayman Chemical Co., Ann Arbor, MI, USA) was dissolved in 50% ethanol. Histamine (Wako Pure Chemical Ind., Osaka) was dissolved in physiological saline. Terfenadine (Sigma, St. Louise, MO, USA) was suspended in 0.5% sodium carboxymethylcellulose. Rabbit polyclonal anti-l-histidine decarboxylase (HDC) antibody (Progen Biotechnik GmbH, Heidelberg, Germany) and rabbit polyclonal anti-β-actin antibody (Abcam, Cambridge, MA, USA) were diluted to the appropriate concentrations with reaction solution 1 (Toyobo Co. Ltd., Osaka). Fluorophore-labeled donkey anti-rabbit IgG antibody
(Invitrogen Co., Carlsbad, CA, USA) was diluted with reaction solution 2 (Toyobo Co. Ltd.). The hair was removed from the rostral part of the back using hair clippers, and 50 μL of 10% sodium laurate solution was applied topically to the shaved skin 3 days later. Histamine (100 nmol/site) was injected intradermally in a volume of 50 μL. Chlorogenic acid was applied topically on the same region in a volume of 50 μL at 90 min after the surfactant application or 30 min before histamine injection; in a series of experiments, chlorogenic acid was applied to the shaved cheek for the evaluation of whether chlorogenic acid itself caused pain or itch (7). Terfenadine (30 mg/kg) was administered orally 30 min before histamine injection.

Mouse behaviors were videotaped with experimenters kept out of the observation room; behavioral observation was performed for 1 h after histamine injection or from 2 h after sodium laurate application; in a series of experiments, behavioral observation was performed for 1 h after chlorogenic acid application to the cheek. Hind-paw scratching of the rostral back was counted during playback of the video; the mouse generally showed several scratchings by its hind-paws for about 1 s and a series of these movements was counted as one bout of scratching (8). In a series of experiments, hind-paw scratching and fore-paw wiping of the cheek were observed as indexes of itch and pain, respectively (7).

For the determination of plasma extravasation, mice were given an intravenous injection of 0.15 mL of 1% Evans blue 20 min before histamine injection. Mice were euthanized by decapitation under sodium pentobarbital anesthesia (80 mg/kg, intraperitoneal) 20 min after histamine injection and the skin was cut out in a circle with a diameter of 8 mm around the injection site. The amount of Evans blue in the skin specimen was determined as described previously (9).

For the determination of histamine and HDC in the epidermis, mice were transcardially perfused with 0.1 M phosphate-buffered saline (PBS) under sodium pentobarbital anesthesia (80 mg/kg, intraperitoneal). The skin was removed from the surfactant-treated region and skin specimens of an 18-mm diameter were taken using a skin punch. The epidermis was separated from the dermis by immersing the skin for 30 s in PBS maintained at 60°C. The epidermis samples were homogenized in 300 μL of lysis buffer (Sigma) using a Precellys 24 tissue homogenizer (Bertin Technologies, Montigny, France) and centrifuged at 10,000 × g for 10 min at 4°C; the supernatant was subjected to histamine and HDC assays. Histamine was determined using a histamine enzyme immunoassay kit (Immunotech, Marseilles, France). For HDC determination, the supernatant (containing 8 μg protein) was electrophoresed on a NuPAGE® 4% – 12% Bis-Tris gel (Invitrogen Co.) and transferred to a polyvinylidene difluoride membrane. After blocking with 1% skim milk in PBS containing 0.1% Tween 20, the membrane was cut at approximately the 50-kDa point, and the upper and lower parts were reacted with rabbit polyclonal anti-HDC and anti-β-actin antibodies (1/1,000 each), respectively, overnight at 4°C. After washing with PBS containing 0.1% Tween 20, the membranes were incubated with fluorophore-labeled donkey anti-rabbit IgG antibody (1/1,000) for 2 h at room temperature. These membranes were then scanned using a fluorescence scanner (Typhoon; GE Healthcare, Munich, Germany) and positive bands were quantified using Scion Image (Scion Corp., Frederick, MD, USA).

Data are presented as means ± standard error of the mean (S.E.M.). Statistical significance was analyzed with two-way repeated measures or one-way analysis of variance followed by Dunnett’s, Tukey’s, or Bonferroni’s test; P < 0.05 was considered significant.

A topical application of 10% sodium laurate significantly increased hind-paw scratching between 2 and
2.5 h after the application, with an increased tendency between 2.5 – 3 h (Fig. 1A); the result was similar to our previous report (3). The effect of chlorogenic acid treatment on the scratching during a period of 2 – 2.5 h after sodium laurate application was examined. Topical post-treatment with 1% and 5% chlorogenic acid inhibited hind-paw scratching induced by 10% sodium laurate in a concentration-dependent manner (Fig. 1B). Topical application of 5% chlorogenic acid alone to the cheek of naïve mice did not increase scratching (scratch bouts/h were 11.0 ± 5.2 and 10.8 ± 2.4, n = 4 each, in vehicle and chlorogenic acid groups, respectively) and did not elicit wiping (n = 4), suggesting that application of chlorogenic acid alone does not induce itch and pain.

Hind-paw scratching and plasma extravasation induced by intradermal injection of histamine (100 nmol/site) were not significantly inhibited by topical application of 5% chlorogenic acid, although they were significantly suppressed by the oral administration of the H1 histamine–receptor antagonist terfenadine (Fig. 2). These results suggest that the inhibitory effect of topical chlorogenic acid on the sodium laurate–induced scratching is not due to the blockade of histamine action.

Systemic administration of chlorogenic acid has been reported to cause partial inhibition of scratching induced by histamine and mast cell degranulation (6). A topical dose of 5% chlorogenic acid might not be enough to exert anti-histamine effects in the dermis. Sodium laurate–induced scratching is not reduced by deficiency in mast cells (3). Therefore, the mast-cell stabilizing activity may not be involved in the inhibitory effect of topical chlorogenic acid on the sodium laurate–induced scratching.

Topical application of 10% sodium laurate increased the content of histamine in the epidermis 2 h after
application, being consistent with our previous report that 10% sodium laurate increased histamine content in the epidermis but not in the dermis (3). Topical post-treatment with 5% chlorogenic acid inhibited the sodium laurate–induced increase of histamine content in the epidermis (Fig. 3A).

Histamine is synthesized mainly by 53-kDa HDC, although the precursor 74-kDa HDC also exhibits low enzyme activity (10). We have shown that sodium laurate exposure increases the ratio of 53-kDa HDC to 74-kDa HDC in the epidermal keratinocytes (3). Thus, we examined the effect of topical application of chlorogenic acid on the sodium laurate action on the HDCs. Topical application of 10% sodium laurate increased 53-kDa HDC in the epidermis 2 h after application, which was significantly inhibited by post-treatment with 5% chlorogenic acid (Fig. 3: B, C). The level of 74-kDa HDC in the epidermis was not affected by topical application of 10% sodium laurate and post-treatment with 5% chlorogenic acid (Fig. 3: B, C). These results suggest that topical administration of chlorogenic acid suppresses the sodium laurate–induced increase of 53-kDa HDC, leading to the inhibition of an increase in histamine production in the epidermal keratinocytes. This may be a mechanism of the inhibitory action of topically applied chlorogenic acid on sodium laurate–induced scratching. The mechanisms of the inhibition of an increase in 53-kDa HDC remain to be investigated.

In conclusion, the present results suggest that topical administration of chlorogenic acid, a constituent of cherry leaves, is effective in the prevention of itching induced by anionic surfactants. Chlorogenic acid is easily dissolved in aqueous solvent and can be added to soap/shampoo. Since chlorogenic acid inhibits the increase in 53-kDa HDC, it is superior to antihistamines for the prevention of anionic surfactant–induced itching.

References